TnT® Quick Coupled Transcription/Translation Systems

Technical Manual No. 045

INSTRUCTIONS FOR USE OF PRODUCTS L1170, L1171, L2080 AND L2081. ***PLEASE DISCARD PREVIOUS VERSIONS.***
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I. Description

The TNT® Quick Coupled Transcription/Translation Systems[a,b,c,d,e] are convenient single-tube, coupled transcription/translation reactions for eukaryotic in vitro translation. The original TNT® Coupled Reticulocyte Lysate Systems[a,b,d,e] simplified the process and reduced the time required to obtain in vitro translation results compared with standard rabbit reticulocyte lysate systems (1). Standard rabbit reticulocyte systems commonly use RNA synthesized in vitro from SP6, T3 or T7 RNA polymerase (1). The TNT® Quick Coupled Transcription/Translation System further simplifies the process by combining the RNA Polymerase, nucleotides, salts and Recombinant RNasin® Ribonuclease Inhibitor[c,e] with the reticulocyte lysate solution to form a single TNT® Quick Master Mix (Figure 1). For most gene constructs, the TNT® Quick reaction produces significantly more protein (2- to 6-fold) in a 60- to 90-minute reaction than a standard in vitro rabbit reticulocyte lysate reaction using RNA templates.

The TNT® Quick Coupled Transcription/Translation System is available in two configurations for transcription and translation of genes cloned downstream from either the T7 or SP6 RNA polymerase promoters. To use these systems, 0.2–2.0µg of circular plasmid DNA containing a T7 or SP6 promoter, or a PCR[f]-generated fragment containing a T7 promoter, is added to an aliquot of the TNT® Quick Master Mix and incubated in a 50µl reaction volume for 60–90 minutes at 30°C. The synthesized proteins are then analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and detected. Included with the TNT® Quick System is a luciferase-encoding control plasmid and Luciferase Assay Reagent[a,g], which can be used in a non-radioactive assay for rapid (<30 seconds) detection of functionally active luciferase protein. Starting with either circular plasmid DNA or PCR-generated DNA, in vitro transcription/translation results may be obtained easily in 5–6 hours.

PCR-Generated fragments are not recommended for use with the SP6 promoter. Use the T7 promoter.

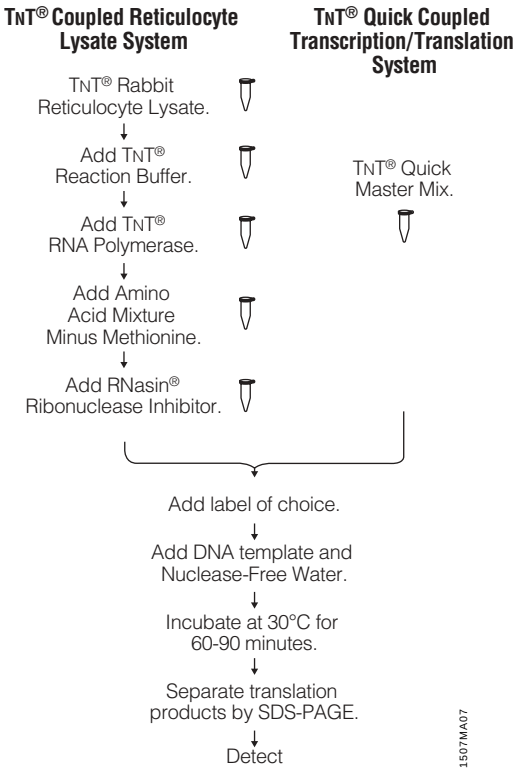
TNT® Coupled Reticulocyte Lysate System
TNT® Quick Coupled Transcription/Translation System
TNT® Rabbit Reticulocyte Lysate.
Add TNT® Reaction Buffer.
Add TNT® RNA Polymerase.
Add Amino Acid Mixture Minus Methionine.
Add RNasin® Ribonuclease Inhibitor.
TNT® Quick Master Mix.
Add label of choice.
Add DNA template and Nuclease-Free Water.
Incubate at 30°C for 60-90 minutes.
Separate translation products by SDS-PAGE.
Detect
1507MA07

Figure 1. Comparison of the TNT® Coupled Reticulocyte Lysate System and the TNT® Quick Coupled Transcription/Translation System protocols.

In addition to verifying the expected molecular weight of a gene construct, the TNT® Quick System is ideal for screening large numbers of constructs for either naturally occurring or deliberately engineered mutations. Applications of the system include:

- Truncation mutation analysis [e.g., the Protein Truncation Test (PTT)] (2)
- Drug screening (affecting translation rates)
- Mutation and detection analysis (i.e., enzyme kinetics)
- Protein-protein interactions (using GST pulldowns)
- Immunoprecipitation of protein complexes
- Protein dimerization assays
- Ligand-binding region determination/ confirmation/competition assays
- In vitro expression cloning (IVEC) (functional genomics)
- Protein structure analysis
- Electrophoretic mobility shift assays (EMSAs) for DNA-protein interactions
- DNA footprinting and protein cross-linking studies
- Protein-RNA binding assays
- Post-translational modification tests
- Verification/characterization of cloned genes

The TNT® Quick Coupled Transcription/Translation Systems are also useful for detecting protein-protein interactions in vitro. Proteins labeled using TNT® Quick Coupled Transcription/Translation System can be used as probes to detect interactions with suspected protein partners that have been expressed as GST- (glutathione-S-transferase), or epitope-tagged fusion proteins (3). [^{35}S]methionine-labeled proteins can be synthesized using coupled in vitro reactions from either full-length cDNAs or deletion mutants. The fusion proteins can be bound to an affinity matrix along with the radioactive proteins with which they interact (4–6). The bound radioactive proteins are then eluted and analyzed by SDS-PAGE or Western analysis (Figure 2; 6). The fusion tag approach has been used to study receptor-mediated control of apoptosis (7).

Alternatively, a non-radioactive approach may be used; the protein is labeled with biotinylated lysine (e.g., Transcend™ Biotinylated tRNA) or is fluorescently tagged (e.g., FluoroTect™ Green$_{Lys}$ BODIPY®-FL -labeled tRNA$^{(h)}$) and combined with a GST-tagged protein. The biotinylated protein is detected by methods similar to those used in Western blotting (8,9).

For a complete list of references for these and other applications, see reference 6 or visit Promega's Technical Resource Center citations at: www.promega.com/citations

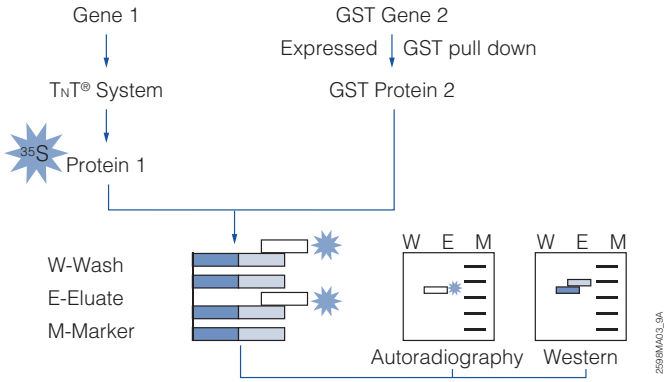

Figure 2. The study of protein-protein interactions using the TNT® Systems (6). This schematic shows translation of one protein with radioactive [^{35}S]methionine in a TNT® System reaction. Large amounts of the suspected partner protein are expressed and purified. A fusion tag (most commonly GST) is incorporated into this second protein to facilitate purification and subsequent capture steps. After the GST fusion protein is immobilized on sepharose (GST pulldowns), it is mixed with the protein produced in the TNT® reaction. The sepharose is washed to remove unbound protein, and the remaining bound proteins are eluted and analyzed on a gel. This technique allows measurement of the protein-protein interactions for both proteins and is often used to verify the in vivo results obtained from yeast two-hybrid experiments.

II. Product Components

Product	Cat.#
TNT® T7 Quick Coupled Transcription/Translation System	L1170
TNT® SP6 Quick Coupled Transcription/Translation System	L2080

For Laboratory Use.
Each system contains sufficient reagents to perform approximately 40 × 50µl translation reactions. Includes:

- 1.6ml TNT® Quick Master Mix (8 × 200µl)
- 5µg SP6 or T7 Luciferase Control DNA (0.5µg/µl)[d]
- 100µl T7 TNT® PCR Enhancer (L1170 only)
- 50µl Methionine, 1mM
- 250µl Luciferase Assay Reagent
- 1.25ml Nuclease-Free Water
- 1 Protocol

Product	Cat.#
TNT® T7 Quick Coupled Transcription/Translation System, Trial Size	L1171
TNT® SP6 Quick Coupled Transcription/Translation System, Trial Size	L2081

For Laboratory Use.
Each system contains sufficient reagents to perform approximately 5 × 50µl translationreactions. Includes:

- 200µl TNT® Quick Master Mix
- 5µg SP6 or T7 Luciferase Control DNA (0.5µg/µl)
- 100µl T7 TNT® PCR Enhancer (L1171 only)
- 50µl Methionine, 1mM
- 1 Protocol

Storage and Stability: Store all components at –70°C. Store the Luciferase Assay Reagent in aliquots; under standard reaction conditions, each control reaction will require 50µl of the Luciferase Assay Reagent. The Luciferase Assay Reagent is stable for 1 month when stored at –20°C or for at least 1 year at –70°C.

Note that the systems are shipped in foil packaging due to sensitivity of the system to carbon dioxide released from dry ice. If storing the system in a freezer containing dry ice, keep system components sealed in foil packaging for best results. **DO NOT** store the unfoiled lysate in the presence of dry ice. Prolonged exposure to dry ice causes significant loss of activity. The expiration date for the TNT® Quick Master Mix is listed on the product vial. **Do not freeze-thaw the Master Mix more than 2 times.** Avoid multiple freeze-thaw cycles or exposure to frequent temperature changes, as these fluctuations can greatly alter product stability.

Do Not store the unfoiled lysate in the presence of dry ice.

III. General Considerations

A. DNA Template Considerations

DNA Expression Elements

1. In addition to circular plasmid DNA, PCR-generated DNA templates can be transcribed/translated using the T7 System. For maximal expression from such templates, it is recommended that approximately 11bp be present upstream of the T7 RNA polymerase promoter for efficient promoter binding. A stop codon (usually UAA) is important for truncated gene products in order to prevent ribosomes from stalling at the ends of RNAs without stop codons. This can be done through appropriate primer design. The best transcription/translation results are obtained when the fragment contains the T7 RNA polymerase promoter. **We do not recommend using linear DNA with the SP6 System because of reduced tanscription efficiencies.**
2. While Rabbit Reticulocyte Lysate-based systems are less sensitive to 5´-untranslated region (UTR) secondary structure than other systems, it is still important to avoid strong hairpin secondary structure in the 5´-UTR region, because this can impair translation efficiency (10).
3. We have observed enhanced translation of proteins when using DNA constructs containing a poly(A) sequence downstream of the gene of interest. Poly(A) sequences are important for mRNA stability and can play a role in translation initiation in Rabbit Reticulocyte Lysate (11). For example, we have observed a 2- to 5-fold increase in the production of luciferase when the gene is cloned into the pSP64 Poly(A) Vector (Cat.# P1241).

Plasmid DNA

1. Residual ethanol should be removed from DNA preparations before they are added to the TNT® Quick Master Mix.
2. Linearized templates produced by restriction enzyme digestion should be cleaned up either by using the Wizard® PCR Preps DNA Purification System(i) or by phenol:chloroform extraction, followed by ethanol precipitation, before use in the TNT® Quick reaction.
3. Plasmid DNA can be purified using the Wizard® *Plus*(j) or Wizard® *Plus* SV(k,l) Minipreps DNA Purification System. DNA prepared by the standard alkaline lysis method described by Sambrook, Fritsch and Maniatis (12) is also sufficiently clean for use in the TNT® Quick Coupled Transcription/Translation

System. For most constructs, optimal results are obtained when 1µg of plasmid DNA template is used. However, we have used 0.2–2.0µg of DNA template and obtained satisfactory levels of translation. The use of more than 1µg of plasmid does not necessarily increase the amount of protein produced.

4. If linearizing plasmid DNA for use with the T7 System, avoid the use of restriction enzymes that yield 3´-overhangs (*Pst* I, *Kpn* I(m), *Sac* I, *Sac* II, *Bst*X I, *Nsi* I, *Apa* I and *Aat* II), as aberrant transcription products can be produced (13). If no alternative enzyme is available, the 3´-overhang can be removed by the addition of Klenow DNA polymerase at a concentration of 5 units/µg DNA followed by incubation for 15 minutes at 22°C.

5. Check the sequence of the DNA template for the presence of additional upstream start codons. During translation, the ribosome is thought to scan from the 5´ end of the RNA and begin translation at the first AUG encountered. Thus, any AUGs within the transcribed portion of the vector or untranslated sequence of the insert may cause translation initiation to occur prior to the desired start codon and result in a shift in the reading frame or production of a larger protein than expected.

PCR-Generated DNA Templates

1. Because PCR DNA templates are usually much smaller than plasmid templates, the amount of DNA necessary for optimal expression is often less than for inserts cloned into plasmid vectors (e.g., for a 500bp PCR product, use 100–800ng for each 50µl TnT® Quick reaction).

2. PCR products can be used directly (up to 7µl) or cleaned up using either the Wizard® PCR Preps DNA Purification System, the Wizard® SV Gel and PCR Clean-Up System or a standard ethanol precipitation and wash.

B. Creating a Ribonuclease-Free Environment

To reduce the chance of RNase contamination, gloves should be worn when setting up experiments, and microcentrifuge tubes and pipette tips should be RNase-free. It is not necessary to add Recombinant RNasin® Ribonuclease Inhibitor to the TnT® Quick reactions to prevent degradation of RNA, because it is already present in the TnT® Quick Master Mix.

C. Handling of Lysate

Except for the actual transcription/translation incubation, all handling of the TnT® Quick Master Mix should be done at 4°C. Any unused Master Mix should be refrozen as soon as possible after thawing to minimize loss of translational activity (see Note 5, Section IV.C). Do not freeze-thaw the Master Mix more than two times.

IV. Translation Procedure

The following is a general guideline for setting up a transcription/translation reaction. Also provided are examples of standard reactions using [^{35}S]methionine (radioactive), Transcend™ Non-Radioactive Detection System (colorimetric or chemiluminescent) or FluoroTect™ $Green_{Lys}$ Systems(h) (fluorescent). Using the Transcend™ Systems, biotinylated lysine residues are incorporated into nascent proteins during translation. This biotinylated lysine is added to the transcription/translation reaction as a precharged ε-labeled, biotinylated lysine-tRNA complex (Transcend™ tRNA) rather than a free amino acid. For more information on the Transcend™ Systems, request

Technical Bulletin #TB182. The FluoroTect™ System uses a charged lysine tRNA labeled with the fluorophore, BODIPY®-FL, to incorporate fluorescently labeled lysine residues into the in vitro translation product. For more information on the FluoroTect™ System, request Technical Bulletin #TB285.

Note: Technical Manuals and Bulletins are available online at: www.promega.com/tbs or by request from Technical Services.

A. General Protocol for TnT® Quick Coupled Transcription/Translation Reactions Using Plasmid DNA

Materials to Be Supplied by the User

- Nuclease-Free Water (Cat.# P1193)
- radiolabeled amino acid (for radioactive detection, Note 4, Seciton IV.C) or Transcend™ tRNA (Cat.# L5061) or Transcend™ Colorimetric (Cat.# L5070) or Chemiluminescent (Cat.# L5080) Translation Detection System (for non-radioactive detection) or FluoroTect™ $Green_{Lys}$ in vitro Translation Labeling System (for fluorescent detection; Cat.# L5001).

1. Remove the reagents from storage at –70°C. Rapidly thaw the TnT® Quick Master Mix by hand-warming and place on ice. The other components can be thawed at room temperature and then stored on ice.
2. Following the example below, assemble the reaction components in a 0.5ml or 1.5ml microcentrifuge tube. After addition of all the components, gently mix by pipetting. If necessary, centrifuge briefly to return the reaction to the bottom of the tube. For additional information on performing a TnT® Quick reaction, see Notes 1–9 in Section IV.C.
3. We recommend including a control reaction containing no added DNA. This reaction allows measurement of any background incorporation of labeled amino acids.

Example of a TnT® Quick Reaction Using Plasmid DNA

Components	Standard Reaction Using [35S]methionine	Standard Reaction Using Transcend™ tRNA	Standard Reaction Using FluoroTect™ $Green_{Lys}$ tRNA
TnT® Quick Master Mix (see Note 3, Section IV.C)	40µl	40µl	40µl
Methionine, 1mM (mix gently prior to use)	–	1µl	1µl
[35S]methionine (1,000Ci/mmol at 10mCi/ml) (see Note 4, Section IV.C)	2µl	–	–
plasmid DNA template(s) (0.5µg/µl) (see Note 6, Section IV.C)	2µl	2µl	2µl
Transcend™ Biotin-Lysyl-tRNA (see Note 9, Section IV.C)	–	1–2µl	–
FluoroTect™ $Green_{Lys}$ tRNA (see Note 9, Section IV.C)	–	–	1–2µl
Nuclease-Free Water to a final volume of	50µl	50µl	50µl

Note: Small-scale reactions may be performed by reducing the recommended volumes proportionally.

4. Incubate the reaction at 30°C for 60–90 minutes.
5. Analyze the results of translation. Procedures for determination of radiolabel incorporation (Section VII.A) and SDS-PAGE analysis of translation products (Section VII.B) are provided.

Note: The optimal coupled transcription/translation reaction occurs in 60–90 minutes at 30°C.

B. General Protocol for TNT® T7 Quick Coupled Transcription/ Translation Reactions Using PCR-Generated DNA

Materials to Be Supplied by the User

- Nuclease-Free Water (Cat.# P1193)
- radiolabeled amino acid (for radioactive detection; Note 4, Seciton IV.C) or Transcend™ tRNA (Cat.# L5061) or Transcend™ Colorimetric (Cat.# L5070) or Chemiluminescent (Cat.# L5080) Translation Detection System (for non-radioactive detection) or FluoroTect™ $Green_{Lys}$ in vitro Translation Labeling System (for fluorescent detection; Cat.# L5001).

1. Remove the reagents from storage at –70°C. Rapidly thaw the TNT® Quick Master Mix by hand-warming and place on ice. The other components can be thawed at room temperature and then stored on ice.
2. Following the example below, assemble the reaction components in a 0.5ml or 1.5ml microcentrifuge tube. After addition of all the components, gently mix by pipetting. If necessary, centrifuge briefly to return the reaction to the bottom of the tube. For additional information on performing a TNT® Quick reaction, see Notes 1–9 in Section IV.C.
3. We recommend including a control reaction containing no added DNA. This reaction allows measurement of any background incorporation of labeled amino acids.

Note: Small-scale reactions may be performed by reducing the recommended volumes proportionally.

Components	Standard Reaction Using [^{35}S]methionine	Standard Reaction Using Transcend™ tRNA	Standard Reaction Using FluoroTect™ $Green_{Lys}$ tRNA
TNT® T7 Quick Master Mix (see Note 3, Section IV.C)	40µl	40µl	40µl
Methionine, 1mM (mix gently prior to use)	–	1µl	1µl
[^{35}S]methionine (1,000Ci/mmol at 10mCi/ml) (see Note 4, Section IV.C)	2µl	–	–
PCR-generated DNA template(s) (see Note 1, Section IV.C)	2.5–5µl	2.5–5µl	2.5–5µl
T7 TNT® PCR Enhancer (see Note 2, Section IV.C)	1µl	1µl	1µl
Transcend™ Biotin-Lysyl-tRNA (see Note 9, Section IV.C)	–	1–2µl	–
FluoroTect™ $Green_{Lys}$ tRNA (see Note 9, Section IV.C)	–	–	1–2µl
Nuclease-Free Water to a final volume of	**50µl**	**50µl**	**50µl**

4. Incubate the reaction at 30°C for 60–90 minutes.
5. Analyze the results of translation. Procedures for determination of radiolabel incorporation (Section VII.A) and SDS-PAGE analysis of translation products (Section VII.B) are provided. If using FluoroTect™ $Green_{Lys}$ tRNA, see Section VII.C; for Transcend™ tRNA reactions, see Section VII.D.

C. Notes

1. PCR-generated templates can be used directly from the amplification reaction. We recommend using 2.5–5µl from the amplification reaction, but up to 7µl can be used in a 50µl reaction. For PCR-generated DNA that has been purified following amplification, we recommend using 100–800ng of the purified product for each reaction.

2. We recommend using 1µl of the T7 TNT® PCR Enhancer in a 50µl reaction to increase transcription/translation when using PCR-generated DNA.

3. The TNT® Quick Master Mix is designed to give the highest expression for most expression constructs. However, we have observed that certain gene constructs may differ in the Mg^{2+} and K^{+} concentrations required for optimal expression in the coupled reaction. For example, some viral leaders will increase translation efficiency and fidelity if additional magnesium acetate and potassium chloride are added to the TNT® Quick reaction. If using a construct with a viral leader, we suggest adding 1–2µl of the T7 TNT® PCR Enhancer.

4. We recommend using a grade of [^{35}S]methionine, such as Amersham Biosciences Redivue™ L-[^{35}S]methionine (Amersham Biosciences Cat.# AG1094), which does not cause the background labeling of the rabbit reticulocyte lysate 42kDa protein. Background labeling of the 42kDa protein can occur using other grades of label (14). In addition, a stabilizer has been added to the Redivue™ [^{35}S]methionine to increase the stability of this product over conventional radiolabeled amino acids, so that the release of volatile gases is reduced substantially. This [^{35}S]methionine may be stored at 4°C without dispensing into aliquots. Other types of ^{35}S-labeled amino acids may be oxidized easily to translation-inhibiting sulfoxides and should be stored in aliquots at –70°C in buffer containing DTT.

   Between 10–40µCi (1–4µl) of [^{35}S]methionine can be added to the TNT® Quick reactions, depending upon the balance between labeling efficiency and cost. For gene constructs that express well and contain several methionines, the 10µCi level (1µl) is sufficient for adequate detection.

5. Except for the actual transcription/translation incubation, all handling of the TNT® Quick System components should be done at 4°C or on ice. Optimum results are obtained when any unused Master Mix is quick frozen with liquid nitrogen as soon as possible after thawing to minimize loss of translational activity.

6. For most plasmid constructs, optimal results are obtained when 1µg of plasmid DNA template is used. We recommend using 0.2–2.0µg of plasmid DNA in TNT® Quick reactions. The use of more than 1µg of plasmid does not necessarily increase the amount of protein produced.

7. Avoid adding calcium to the transcription/translation reaction. Calcium may reactivate the micrococcal nuclease used to destroy endogenous RNA in the Master Mix and result in degradation of DNA or RNA templates.

8. The TNT® Quick Master Mix contains roughly 100–200mg/ml of endogenous protein.

9. The level of added Transcend™ tRNA and FluoroTect™ $Green_{Lys}$ tRNA can be increased (1–4µl) to allow more sensitive detection of proteins that contain few lysines or are poorly expressed.

V. Positive Control Translation Reactions Using Luciferase

The assay for firefly luciferase activity is extremely sensitive, rapid and easy to perform. It is a good control for in vitro translations because only full-length luciferase is active. Additionally, luciferase is a monomeric protein (61kDa) that does not require post-translational processing or modification for enzymatic activity. Promega's Luciferase Assay System[a,g] is a substantial improvement over conventional methods in both sensitivity and simplicity (15).

The control reaction can be performed with or without the addition of radiolabeled amino acids.

A. Radioactive Luciferase Control Reaction

1. The following example contains [^{35}S]methionine:

TNT® Quick Master Mix (see Note 3, Section IV.C)	40µl
[^{35}S]methionine (1,000Ci/mmol at 10mCi/ml) (see Note 4, Section IV.C)	2µl
Appropriate Luciferase Control DNA (0.5µg/µl) (see Section XI.B)	2µl
Nuclease-Free Water to a final volume of	50µl

2. Incubate the reaction at 30°C for 60–90 minutes (see Note 3, Section IV.C).
3. Analyze the results of translation by measuring direct incorporation of radiolabel (Section VII.A) and/or gel analysis of translation products (Section VII.B).
4. The Luciferase Control reactions can be stored at –20°C for up to 2 months or at –70°C for up to 6 months with little loss of luciferase activity.

B. Non-Radioactive Luciferase Control Reaction

1. The following example contains Methionine:

TNT® Quick Master Mix (see Note 3, Section IV.C)	40µl
Methionine, 1mM	1µl
Appropriate Luciferase Control DNA (0.5µg/µl) (see Section XI.B)	2µl
Nuclease-Free Water to a final volume of	50µl

2. Incubate the translation reaction at 30°C for 60–90 minutes.
3. Test for the synthesis of functional luciferase using the standard luciferase assay (see Section VIII.A).
4. The Luciferase Control reactions can be stored at –20°C for up to 2 months or at –70°C for up to 6 months with little loss of luciferase activity.

For Assistance...
in troubleshooting Microsomal Membrane translation reactions, contact Promega's Technical Services.
Email: techserv@promega.com

VI. Cotranslational Processing Using Canine Pancreatic Microsomal Membranes

Microsomal vesicles are used to study cotranslational and initial post-translational processing of proteins. Processing events such as signal peptide cleavage, membrane insertion, translocation and core glycosylation can be examined by the translation of the appropriate gene in vitro in the presence of these membranes. To ensure consistent performance with minimal background, Promega's Canine Pancreatic Microsomal Membranes[e] (Cat.# Y4041) have been isolated so that they are free from mRNA.

Materials to Be Supplied by the User

- Canine Pancreatic Microsomal Membranes (Cat.# Y4041)
- [^{35}S]methionine (1,000Ci/mmol at 10mCi/ml))

A. General Protocol for Translation with Microsomal Membranes

1. Remove the reagents from the freezer and allow them to thaw on ice.
2. Mix the following components on ice, in the order given, in a sterile 1.5ml microcentrifuge tube:

T7 TNT® Quick Master Mix	20µl
[^{35}S]methionine (1,000Ci/mmol at 10mCi/ml) (see Note 4, Section IV.C)	2.0µl
plasmid DNA, 0.5µg	0.5µl
Canine Pancreatic Microsomal Membranes (see Note 1, below)	0.3–1.8µl
Nuclease-Free Water to a final volume of	25µl

3. Incubate at 30°C for 60–90 minutes.
4. Analyze the results of translation and processing. Procedures for incorporation assays (Section VII.A) and SDS-PAGE analysis of translation products (Section VII.B) are provided.

Notes:

1. The amount of Canine Microsomal Membranes used in the reaction may need to be titrated. While these reaction conditions will be suitable for most applications, the efficiency of processing using membranes may vary. Thus, reaction parameters may need to be altered to suit individual requirements. In general, increasing the amount of membranes in the reaction increases the proportion of polypeptides processed but reduces the total amount of polypeptides synthesized.
2. For reactions using the TNT® Quick CoupledTranscription/Translation System, the Canine Microsomal Membranes will inhibit transcription. We do not recommend exceeding 1.8µl of Canine Microsomal Membranes. Transcription/Translation may be inhibited by as much as 50% with 0.6µl of Canine Microsomal Membranes.
3. The amount of protein produced in TNT® Quick reactions using Canine Pancreatic Microsomal Membranes will be less than the amount produced in TNT® Quick reactions alone. Depending on the construct used, protein synthesis efficiency can be expected to drop between 10–50% in the presence of Microsomal Membranes.
4. In some cases, it is difficult to determine if efficient processing or glycosylation has occurred by gel analysis alone. Other assays, such as various protection assays (16), may be required to determine if processing events have taken place.

Note: The storage buffer for Canine Pancreatic Microsomal Membranes is 50mM triethanolamine, 2mM DTT and 250mM sucrose.

Note: TNT® Quick Coupled Transcription/ Translation Systems are not tested for performance with Canine Microsomal Membranes.

Note: We do NOT recommend using Canine Microsomal Membranes when using SP6 TNT® Quick Coupled Transcription/ Translation Systems, because SP6 polymerase is sensitive to salts. Transcription may be inhibited as much as 70% by the presence of Canine Microsomal Membranes in the reaction.

VII. Post-Translational Analysis

Materials to Be Supplied by the User
(Solution compositions are provided in Section XI.A.)

- 1M NaOH
- 25% TCA/2% casamino acids (Difco® brand, Vitamin Assay Grade)
- 5% TCA
- Whatman® GF/A glass fiber filter (Whatman® Cat.# 1820 021)
- acetone
- Whatman® 3MM filter paper
- 30% acrylamide solution
- separating gel 4X buffer
- stacking gel 4X buffer
- SDS sample buffer
- SDS polyacrylamide gels
- **optional:** precast polyacrylamide gels

A. Determination of Percent Incorporation of Radioactive Label

1. After the 50µl translation reaction is complete, remove 2µl from the reaction and add it to 98µl of 1M NaOH/2% H_2O_2.
2. Vortex briefly and incubate at 37°C for 10 minutes.
3. At the end of the incubation, add 900µl of ice-cold 25% TCA/2% casamino acids to precipitate the translation product. Incubate on ice for 30 minutes.
4. Wet a Whatman® GF/A glass fiber filter with a small amount of ice-cold 5% TCA. Collect the precipitated translation product by vacuum filtering 250µl of the TCA reaction mix. Rinse the filter 3 times with 1–3ml of ice-cold 5% TCA. Rinse once with 1–3ml of acetone. Allow the filter to dry at room temperature or under a heat lamp for at least 10 minutes.
5. For determination of ^{35}S incorporation, put the filter in the appropriate scintillation cocktail, invert to mix and count in a liquid scintillation counter.
6. To determine total counts present in the reaction, spot a 5µl aliquot of the TCA reaction mix directly onto a filter. Dry the filter for 10 minutes. Count in a liquid scintillation counter as in Step 5.
7. To determine background counts, remove 2µl from a 50µl translation reaction containing no DNA and proceed as described in Steps 1–5.
8. Perform the following calculation to determine percent incorporation:

$$\frac{\text{cpm of washed filter (Step 5)}}{\text{cpm of unwashed filter (Step 6)} \times 50} \times 100 = \text{percent incorporation}$$

9. Perform the following calculation to determine the fold stimulation over background:

$$\frac{\text{cpm of washed filter (Step 5)}}{\text{cpm of “no DNA control reaction” filter (Step 7)}} = \text{fold stimulation}$$

B. Denaturing Gel Analysis of Radioactively Labeled Translation Products

Precast polyacrylamide gels are available from a number of manufacturers. For protein analysis, NOVEX™ and Bio-Rad® Laboratories, Inc., offer a variety of precast mini-gels, which are compatible with their vertical electrophoresis and blotter systems. These companies offer Tris-Glycine, Tricine and Bis-Tris gels for resolution of proteins under different conditions and over a broad spectrum of protein sizes. The NOVEX™ 4–20% Tris-Glycine gradient gels (NOVEX™ Cat.#

EC6025 or EC60355) and the Bio-Rad® Ready Gel 4–20% Tris-Glycine Gel, 10-well (Bio-Rad® Cat.# 161-0903) are convenient for resolving proteins over a wide range of molecular weights. In addition to convenience and safety, precast gels provide consistent results.

1. Once the 50µl translation reaction is complete (or at any desired timepoint), remove a 1–5µl aliquot and add it to 20µl of SDS sample buffer. The remainder of the reaction may be stored at –20°C, or at –70°C for long-term storage.
2. Cap the tube and heat at 100°C for 2 minutes to denature the proteins. This may cause protein aggregation. A 70°C incubation for 10 minutes may be more appropriate for some samples.
3. A small aliquot (5–10µl) of the denatured sample can then be loaded onto an SDS-polyacrylamide gel or stored at –20°C. It is not necessary to separate labeled polypeptides from free amino acids by acetone precipitation.
4. Typically, electrophoresis is carried out at a constant current of 15mA in the stacking gel and 30mA in the separating gel (or 30mA for a gradient gel). Electrophoresis is usually performed until the bromophenol blue dye has run off the bottom of the gel. Disposal of unincorporated label may be easier if the gel is stopped while the dye front remains in the gel, as the dye front also contains the unincorporated labeled amino acids. If transferring the gel to a membrane filter for Western blotting, proceed to Step 7.
5. Place the polyacrylamide gel in a plastic box and cover the gel with fixing solution (as prepared in Section XI.A) for 30 minutes. Agitate slowly on an orbital shaker. Pour off the fixing solution. Proceed to Step 6 (gel drying prior to film exposure).

   Optional: Labeled protein bands in gels may be visualized by autoradiography or fluorography. Fluorography dramatically increases the sensitivity of detection of ^{35}S-, ^{14}C- and ^{3}H-labeled proteins and is recommended for the analysis of in vitro translation products. The increased detection sensitivity of fluorography is obtained by infusing an organic scintillant into the gel. The scintillant converts the emitted energy of the isotope to visible light and increases the proportion of energy that may be detected by X-ray film. Commercial reagents, such as Amplify™ Reagent (Amersham Biosciences), can be used for fluorographic enhancement of signal. Alternatively, the fixed gel can be exposed to a phosphorimaging screen. These systems provide greater sensitivity, greater speed and the ability to quantitate the radioactive bands.
6. Dry the gel before exposure to film as follows: Soak the gel in 7% acetic acid, 7% methanol, 10% glycerol for 5 minutes to prevent the gel from cracking during drying. Place the gel on a sheet of Whatman® 3MM filter paper, cover with plastic wrap and dry at 80°C for 30–90 minutes under a vacuum using a conventional gel dryer; dry completely. The gel also may be dried overnight using Promega's Gel Drying Kit (Cat.# V7120). To decrease the likelihood of cracking gradient gels, dry them with the wells pointing down. Expose the gel on Kodak X-OMAT® AR film for 1–6 hours at –70°C (with fluorography), or 6–15 hours at room temperature (with autoradiography).
7. For Western blot analysis of proteins, transfer (immobilize) the protein from the gel onto nitrocellulose or PVDF membrane (17,18). Usually Western blots are made by electrophoretic transfer of proteins from SDS-polyacrylamide gels. Detailed procedures for electrophoretic blotting usually are

included with commercial devices and can be found in references 17, 19, 20 and 21. A general discussion of Western blotting with PVDF membranes is found in reference 22. PVDF membranes must be prewet in methanol or ethanol before equilibrating in transfer buffer. The blot may then be subjected to immunodetection analysis. For more information, refer to Promega's *Protocols and Applications Guide*, Third Edition (23).

C. Denaturing Gel Analysis of Translation Products Labeled with the FluoroTect™ Green$_{Lys}$ in vitro Translation Labeling System

Note: The use of gel systems other than tris-glycine may cause different migration patterns for the expressed and background bands.

The fluorescent translation product should be resolved by running a sample on an SDS-PAGE and then visualized by placing the gel on a laser-based fluorescence scanning device.

Denaturing Gel Analysis

1. Once the translation reaction is complete (or at any desired time point), remove a 5µl aliquot and add it to 20µl of 1X SDS gel-loading buffer. Store the remainder of the translation reaction at –20°C. The FluoroTect™ tRNA fluorophore is sensitive to extreme heating. If heating to denature the proteins, do not exceed 70°C for more than 2–3 minutes.
2. Load the sample from Step 1 on an SDS-PAGE gel.
3. Peform electrophoresis using standard conditions for your apparatus. Typically, electrophoresis is carried out at a constant current of 20mA. Electrophoresis usually is performed until the bromophenol blue dye has run to the bottom of the gel.

Note: The Storm® instrument (Molecular Dynamics) is not recommended for use with the FluoroTect™ System due to reduced sensitivity.

Fluorescent Detection

Materials to Be Supplied by the User

- Fluorescent Imaging Instrument (i.e., FluorImager® SI or FluorImager® 595 [Molecular Dynamics], both with a 499 argon laser; the Typhoon™ 8600 [Molecular Dynamics], with a 532nm excitation, or the FMBIO® II [Hitachi}, with a 505 channel)

After electrophoresis is completed, immediately place the gel in water and then complete fluorescent scanning.

Use gloves ... when handling the gels.

Notes:

1. Fixing polyacrylamide gels does not interfere with the detection of FluoroTect™ Green$_{Lys}$-labeled in vitro **translation products,** although the signal intensity may be somewhat decreased.
2. Drying fixed polyacrylamide gels in cellophane does not interfere with the detection of FluoroTect™ Green$_{Lys}$-labeled in vitro translation products, although signal intensity may be somewhat decreased.
3. Fixing and/or drying gels may decrease the signal intensity of **prestained molecular weight markers**, making them difficult to detect with fluorescent scanners.

Immunoprecipitation and Western Blot Analysis

Anti-BODIPY®-FL is available from Molecular Probes (Molecular Probes Cat.# A-5770) for immunoprecipitation and western blot analysis of translation products.

D. Denaturing Gel Analysis of Translation Products Labeled Using the Transcend™ Non-Radioactive Translation Detection Systems

Biotinylated protein standards (Bio-Rad Cat.# 161-0319) can be used to determine the apparent molecular weight of the translated biotinylated protein. Alternatively, fluorescently labeled size standards can be observed after transfer and marked with a pencil under UV irradiation. The positions of unlabeled size standards also can be determined by staining the blot after transfer (See *Transcend™ Non-Radioactive Translation Detection Systems Technical Bulletin* #TB182).

1. Once the 50µl translation reaction is complete (or at any desired time point), remove a 1µl aliquot and add it to 15µl of SDS sample buffer. The remainder of the reaction may be stored at –20°C.
2. Close the tube and heat at 90–100°C for 2 minutes to denature the proteins.

   Note: In some cases, high molecular weight complexes are formed at 100°C, and denaturation may need to be performed at lower temperatures (e.g., 20 minutes at 60°C or 3–4 minutes at 80–85°C).
3. Load the denatured sample on an SDS-polyacrylamide gel. (Protocols for SDS polyacrylamide gel electrophoresis may be found in Promega's *Protocols and Applications Guide* (23).
4. Perform electrophoresis using standard conditions for your apparatus. Typically, electrophoresis is carried out at a constant current of 20mA. Electrophoresis usually is performed until the bromophenol blue dye has run off the bottom of the gel.

   Note: If a gene product is weakly expressed or contains few lysines, up to 2µl of the translation reaction (Reticulocyte Lysate) can be loaded on an SDS gel without the loss of resolution observed with autoradiography. However, loading more of the translation reaction can result in high background on the blot.

Electroblotting of Proteins to Membrane

For colorimetric detection see *Transcend™ Non-Radioactive Translation Detection Systems Technical Bulletin* #TB182, Section V.C. The translation products can be blotted from the SDS-polyacrylamide gel to (in decreasing order of preference) PVDF, nitrocellulose or another membrane using any standard apparatus and protocol, including semi-dry systems. Detailed procedures for electrophoretic blotting are usually included with commercial devices. We routinely transfer at a constant voltage of 100V for 60 minutes using a minigel-size electroblotting unit or 15 minutes using a semi-dry system. PVDF membrane must be pre-wet in methanol before it is equilibrated in transfer buffer.

Instructions for chemiluminescent detection of products are found in the *Transcend™ Non-Radioactive Translation Detection Systems Technical Bulletin* #TB182, Section V.D.

VIII. Positive Control Luciferase Assays

The Luciferase Assay Reagent and samples should be at ambient temperature prior to performing a luciferase assay.

Light intensity is a measure of the rate of catalysis by luciferase and is therefore dependent upon temperature. The optimum temperature for luciferase activity is approximately room temperature (20–25°C). It is important that the Luciferase Assay Reagent be fully equilibrated to room temperature before beginning measurements. To ensure temperature equilibration, place a thawed aliquot of the Luciferase Assay Reagent in a sealed tube into a water bath maintained at ambient temperature, and equilibrate for at least 30 minutes. The sample to be assayed should also be at ambient temperature.

Either a luminometer or a scintillation counter can be used for quantitation. (There is usually insufficient light output for qualitative visual detection.) A luminometer can measure as little as 10^{-20} moles (0.001pg) of luciferase, whereas a scintillation counter typically has a less sensitive detection limit. However, the limits of sensitivity may vary depending upon the particular instrument used. The assay should be linear in some portion of the detection range of the instrument. Please consult your instrument operator's manual for general operating instructions.

A. Using a Luminometer

1. Dispense 50µl of the Luciferase Assay Reagent into luminometer tubes, one tube per sample.
2. Program the luminometer to perform a 2-second measurement delay followed by a 10-second measurement read for luciferase activity. The read time may be shortened if sufficient light is produced.
3. Add 2.5µl of cell lysate to a luminometer tube containing the Luciferase Assay Reagent. Mix by pipetting 2–3 times or vortex briefly.
4. Place the tube in the luminometer and initiate reading.
5. If the luminometer is not connected to a printer or computer, record the reading.

Do not add scintillant, because it will inactivate the luciferase and is not needed.

B. Using a Scintillation Counter

Ideally, the coincidence circuit of the scintillation counter should be turned off. Usually, this is achieved through an option of the programming menu or by a switch within the instrument. Consult the user's manual or the manufacturer of the scintillation counter. If the circuit cannot be turned off, a linear relationship between luciferase concentration and cpm still can be produced by calculating the square root of measured counts per minute (cpm) minus background cpm (i.e., $[\text{sample} - \text{background}]^{1/2}$). To measure background cpm, use water or Luciferase Assay Reagent as a blank.

Use the same protocol as luciferase assays using a luminometer (Section VII.A). The sample may be placed directly in the scintillation vial if it completely covers the bottom of the vial (clear or translucent vials are acceptable). Do not add scintillant, because it will inactivate luciferase. Alternatively, place the sample in a microcentrifuge tube, and then place the tube in the scintillation vial. To ensure consistency when working with multiple samples, place each microcentrifuge tube at the same relative position within the scintillation vial.

For consistency in measuring luciferase activity, use the scintillation counter in manual mode. Initiate each sample reaction immediately before measurement, and read the samples one at a time. Because the enzymatic reaction produces light at all wavelengths, read the samples with all channels open (open window). To reduce background counts, it may be necessary to wait 10–30 seconds before counting. Read individual samples for 1–5 minutes.

IX. Troubleshooting

Symptoms	Possible Causes	Comments
The control reaction produces no luciferase	Loss of activity of reaction components	The lysate should not be used after more than two freeze-thaw cycles. Do not use reagents after the expiration date.
	Ethanol or salt present in the translation reaction	Ethanol or salt may inhibit translation.
Low translation efficiency	Certain gene constructs may require different Mg^{2+} and K^+ concentrations for optimal expression	Add 1–3µl of the T7 TNT® PCR Enhancer.
	Calcium is present in the translation reaction	Avoid adding calcium to the translation reaction. Calcium may reactivate the micrococcal nuclease used used to destroy endogenous mRNA in the lysate and result in degradation of the DNA or mRNA template.
	Ethanol present in the translation reaction	Residual ethanol should be removed from template DNA preparations and amino acids before they are added to the translation reaction.
	Incubation of the reaction at 37°C causes decreased protein synthesis	Incubate the reaction at 30°C.
Unexpected bands present at higher molecular weights or bands stuck in stacking gel.	Denaturing temperature is too high	Denature sample at a lower temperature (e.g., 60–80°C) for 10–15 minutes.
Unexpected bands present on the gel	Proteolysis of translation product	Add protease inhibitors, such as α2-macroglobulin, leupeptin or chymostatin (0.5–1µg/ml).
	More than one peptide is translated from the template	Leaky scanning for translation initiation can result in translation initiating at internal methionines. Optimizing the Mg^{2+} or K^+ concentration can increase fidelity (24).
	The [^{35}S]methionine used is not of translational grade or beyond its expiration date	There are reports of a 42kDa band with some grades of [^{35}S]methionine (14). We recommend Amersham Biosciences Redivue™ L-[^{35}S]methionine (Amersham Biosciences Cat.# AG1094) to avoid this 42kDa band.
	Globin may appear on the autoradiogram or stained gel	Globin may show on a stained gel and occasionally on the autoradiogram. It appears as a broad band migrating at 10–15kDa.

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: **www.promega.com**.

E-mail: **techserv@promega.com**

Troubleshooting (continued)

Symptoms	Possible Causes	Comments
Unexpected bands present on the gel (continued)	Aminoacyl tRNAs may produce background bands (~25kDa)	Add RNase A to the lysate reaction (after completion) to a final concentration of 0.2mg/ml. Incubate for 5 minutes at 30°C.
	Oxidized β-mercapto-ethanol is present or not enough SDS in the loading buffer	Use a loading buffer that contains 2% SDS and 100mM DTT.
There is smearing on the gel	Gel not clean	Gel must be washed before placing onto film. Once gel electrophoresis is complete, soak the gel in either a standard Coomassie® destaining solution (50% methanol, 7.5% glacial acetic acid) or in water for 15–30 minutes prior to drying.
	Too much protein loaded on the gel	Check the amount of samples loaded on the gel and the amount of loading buffer. Too much protein loaded can cause smearing.
	Acrylamide concentration in the gel is too low	Acrylamide concentration can be increased to 12%.
	Sample contains ethanol	Ethanol can cause gel smearing.

X. References

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XI. Appendix

A. Composition of Buffers and Solutions

acrylamide solution, 30%

30g	acrylamide
0.8g	bisacrylamide

Add water to a final volume of 100ml. Store at 4°C.

fixing solution

50%	methanol
10%	glacial acetic acid
40%	water

1X SDS gel-loading buffer

50mM	Tris-HCl (pH 6.8)
100mM	dithiothreitol
2%	SDS
0.1%	bromophenol blue
10%	glycerol

1X SDS gel-loading buffer lacking dithiothreitol can be stored at room temperature. **Dithiothreitol should be added from a 1M stock just before the buffer is used.**

SDS polyacrylamide running 10X buffer

30g	Tris base
144g	glycine
100ml	10% SDS

Add deionized water to a final volume of 1 liter. Store at room temperature.

separating gel 4X buffer

18.17g	Tris base
4ml	10% SDS

Bring the volume to approximately 80ml with deionized water. Adjust to pH 8.8 with 12N HCl and add deionized water to a final volume of 100ml. Store at room temperature.

stacking gel 4X buffer

6.06g	Tris base
4ml	10% SDS

Bring the volume to approximately 80ml with deionized water. Adjust to pH 6.8 with 12N HCl and add deionized water to a final volume of 100ml. Store at room temperature.

B. Luciferase SP6/T7 Control DNAs

The Luciferase SP6/T7 Control DNAs are used as functional controls in the TNT® Quick Coupled Transcription/Translation System. The Control DNAs contain the gene for luciferase under transcriptional control of a phage RNA polymerase promoter. The constructs carry a 30bp poly[d(A)/d(T)] tail following the luciferase gene. The maps of the Luciferase SP6 Control DNA and T7 Control DNA are shown in Figures 3 and 4, respectively. Please note that these vectors are intended for use as control luciferase expression vectors only. They are not intended for use as cloning vectors.

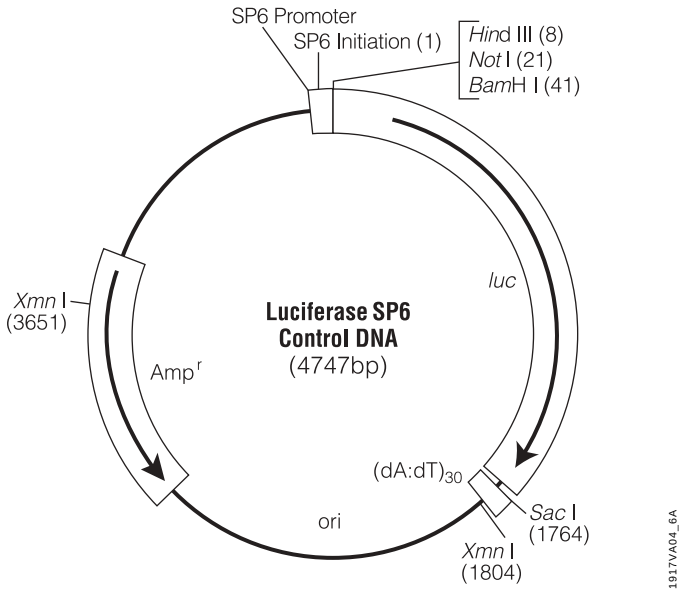

Figure 3. Luciferase SP6 Control DNA circle map and sequence reference points. Additional description: Amp^r, β-lactamase gene (resistant to ampicillin); ori, origin of plasmid replication.

Sequence reference points:	
SP6 RNA polymerase initiation	1
GL Primer 2	49–71
Luciferase gene	48–1700
Poly(A) (dA)30	1767–1796
pUC/M13 reverse primer (17mer)	1833–1817
pUC/M13 reverse primer (22mer)	1838–1817
β-lactamase gene (Ampr)	3838–2978
SP6 RNA polymerase promoter primer	4731–1
SP6 RNA polymerase promoter	4731–3

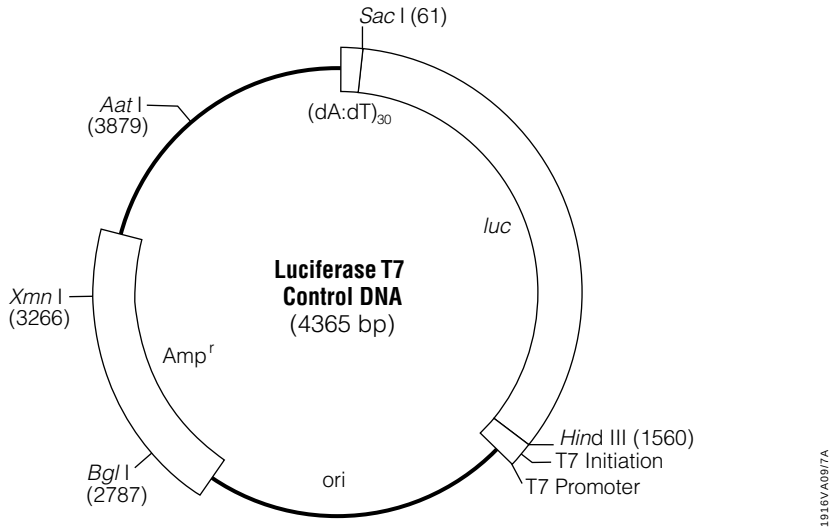

Figure 4. Luciferase T7 Control DNA circle map and sequence reference points. Additional description: Amp^r, β-lactamase gene (resistant to ampicillin); ori, origin of plasmid replication.

Sequence reference points:	
T7 RNA polymerase initiation	1
Gl Primer 2	52–74
Luciferase gene	51–1700
Poly(A) $(dA)_{30}$	1770–1799
β-lactamase gene (Amp^r)	2444–3304
T7 RNA polymerase promoter	4315–3
T7 RNA polymerase promoter Primer	4315–3

C. Related Products

General Considerations

The in vitro synthesis of proteins is a popular method in biological research. Among other applications, translation systems are used to rapidly characterize plasmid clones, study structural mutations and examine translational signals.

Two basic approaches to in vitro protein synthesis are available: 1) systems programmed with RNA (translation systems), or 2) systems programmed with DNA (coupled transcription/translation systems). Several general considerations to assist you in selecting the appropriate Promega product(s) are discussed in this section.

Translation Systems

A number of cell-free translation systems have been developed for the translation of mRNA isolated from tissue or generated in vitro. Promega offers several Rabbit Reticulocyte Lysate(a,d,e) and Wheat Germ Extract Systems. All are reliable, convenient and easy-to-use systems to initiate translation and produce full-size polypeptide products. Rabbit Reticulocyte Lysate is appropriate for the translation of larger mRNA species and generally is recommended when microsomal membranes are to be added for cotranslational processing of translation products. Promega's Flexi® Rabbit Reticulocyte Lysate(a,d,e) is recommended where optimization of translation of particular RNAs through adjustments to salt and DTT concentrations is required. Wheat Germ

Extract is recommended for translation of RNA preparations containing low concentrations of double-stranded RNA (dsRNA) or oxidized thiols, which are inhibitory to reticulocyte lysate.

Coupled Transcription/Translation Systems

DNA sequences cloned in plasmid vectors also may be expressed directly using either Promega's TnT® Coupled Reticulocyte Lysate Systems, Wheat Germ Extract Systems or *E. coli* S30 Extract Systems[a,d]. The TnT® Systems are used to direct eukaryotic translation, whereas the S30 Systems are under prokaryotic translational controls. The TnT® Systems require plasmid constructs containing a phage RNA polymerase promoter (SP6, T3 or T7) for the initiation of transcription, but translation in this system is under eukaryotic controls. Optimal translation will occur if the AUG initiation codon is in a "Kozak consensus" context (A/GCCAUGG) (25) in the absence of inhibiting secondary structure. The template DNA to be expressed in the S30 Systems must contain *E. coli* promoter sequences or a phage T7 promoter sequence and prokaryotic ribosome binding sites (GGAGG) for translation. The TnT® and *E. coli* S30 Systems can use either circular or linear DNA templates.

TnT® Coupled Reticulocyte Lysate Systems

Product	Size	Cat.#
TnT® SP6 Coupled Reticulocyte Lysate System[a,b,d,e]	40 reactions	L4600
TnT® T7 Coupled Reticulocyte Lysate System[a,b,d,e]	40 reactions	L4610
TnT® T3 Coupled Reticulocyte Lysate System[a,b,d,e]	40 reactions	L4950
TnT® T7/T3 Coupled Reticulocyte Lysate System[a,b,d,e]	40 reactions	L5010
TnT® T7/SP6 Coupled Reticulocyte Lysate System[a,b,d,e]	40 reactions	L5020
TnT® T7 Quick for PCR DNA[b,c,e]	40 reactions	L5540
TnT® SP6 Coupled Reticulocyte Lysate System, Trial Size[a,b,d,e]	8 reactions	L4601
TnT® T7 Coupled Reticulocyte Lysate System, Trial Size[a,b,d,e]	8 reactions	L4611

For Laboratory Use.

TnT® Coupled Wheat Germ Extract Systems

Product	Size	Cat.#
TnT® T3 Coupled Wheat Germ Extract System[a,b,d,e]	40 reactions	L4120
TnT® SP6 Coupled Wheat Germ Extract System[a,b,d,e]	40 reactions	L4130
TnT® T7 Coupled Wheat Germ Extract System[a,b,d,e]	40 reactions	L4140
TnT® T7/SP6 Coupled Wheat Germ Extract System[a,b,d,e]	40 reactions	L5030
TnT® T7/T3 Coupled Wheat Germ Extract System[a,b,d,e]	40 reactions	L5040

For Laboratory Use.

Promega

Rabbit Reticulocyte Lysate

Product	Size	Cat.#
Rabbit Reticulocyte Lysate, Nuclease-Treated*[a,d,e]	30 reactions	L4960
Rabbit Reticulocyte Lysate, Untreated	1ml	L4151

*For Laboratory Use.
Bulk Rabbit Reticulocyte Lysate is available from Promega.

Flexi® Rabbit Reticulocyte Lysate System

Product	Size	Cat.#
Flexi® Rabbit Reticulocyte Lysate System[a,d,e]	30 reactions	L4540

Bulk Flexi® Rabbit Reticulocyte Lysate is available from Promega.

Wheat Germ Extract

Product	Size	Cat.#
Wheat Germ Extract	30 reactions	L4380

Rabbit Reticulocyte Lysate/Wheat Germ Extract Combination System

Product	Size	Cat.#
Rabbit Reticulocyte/Wheat Germ Extract Combination System	12 reactions	L4330

E. coli S30 Extract Systems

Product	Size	Cat.#
E. coli S30 Extract for Linear Templates[a,d]	30 reactions	L1030
E. coli S30 Extract for Circular DNA[a,d]	30 reactions	L1020
E. coli T7 S30 Extract System for Circular DNA	30 reactions	L1130

Transcend™ Non-Radioactive Translation Detection Systems

Product	Size	Cat.#
Transcend™ Colorimetric Translation Detection System	30 reactions	L5070
Transcend™ Chemiluminescent Non-Radioactive Translation Detection System (Colorimetric)	30 reactions	L5080
Transcend™ Biotinylated tRNA	30µl	L5061

For Laboratory Use.

FluoroTect™ $Green_{Lys}$ in vitro Translation Labeling System

Product	Size	Cat.#
FluoroTect™ $Green_{Lys}$ in vitro Translation Labeling System[h]	40 reactions	L5001

For Laboratory Use.

Canine Pancreatic Microsomal Membranes

Product	Size	Cat.#
Canine Pancreatic Microsomal Membranes[e]	50µl	Y4041

(a)U.S. Pat. Nos. 5,283,179, 5,641,641, 5,650,289 and 5,814,471, Australian Pat. No. 649289, European Pat. No. 0 553 234, and Japanese Pat. No. 317595 have been issued to Promega Corporation for a firefly luciferase assay method, which affords greater light output with improved kinetics as compared to the conventional assay. Other patents are pending.

(b)U.S. Pat. Nos. 5,324,637, 5,492,817 and 5,665,563, European Pat. No. 0 566 714 B1, Australian Pat. No. 660329 and Japanese Pat. No. 2904583 have been issued to Promega Corporation for coupled transcription/translation systems that use RNA polymerases and eukaryotic lysates.

(c)U.S. Pat. No. 5,552,302, European Pat. No. 0 422 217, Australian Pat. No. 646803, and Japanese Pat. No. 3009458 have been issued to Promega Corporation for the methods and compositions for production of human recombinant placental ribonuclease inhibitor.

(d)The method of recombinant expression of *Coleoptera* luciferase is covered by U.S. Pat. Nos. 5,583,024, 5,674,713 and 5,700,673.

(e)U.S. Pat. Nos. 4,966,964, 5,019,556 and 5,266,687, and Australian Pat. Nos. 616881 and 641261 and other pending and issued patents, which claim vectors encoding a portion of human placental ribonuclease inhibitor, are exclusively licensed to Promega Corporation.

(f)The PCR process is covered by patents issued and applicable in certain countries. Promega does not encourage or support the unauthorized or unlicensed use of the PCR process.

(g)Certain applications of this product may require licenses from others.

(h)FluoroTect™ $Green_{Lys}$ incorporates dye conjugates made with the BODIPY®-FL fluorescent reactive dyes, which are licensed from Molecular Probes, Inc., under U.S. Pat. Nos. 4,774,339, 5,274,113 and 5,433,896 for IVE analysis for research use only including GPR and ASR applications and Fluorotag® technology. BODIPY is a registered trademark of Molecular Probes, Inc, and Fluorotag is a registered trademark of AmberGen, Inc.

(i)Licensed under U.S. Pat. No. 5,075,430.

(j)U.S. Pat. Nos. 5,658,548 and 5,808,041, and Australian Pat. No. 689815 have been issued to Promega Corporation for nucleic acid purification on silica gel and glass mixtures. Other patents pending.

(k)U.S. Pat. No. 5,981,235 and Australian Pat. No. 729932 have been issued to Promega Corporation for methods for isolating nucleic acids using alkaline protease. Other patents are pending.

(l)Australian Pat. No. 730718 and Singapore Pat. No. 64532 has been issued to Promega Corporation for an improved filtration system and method. Other patents are pending.

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Product claims are subject to change. Please contact Promega Technical Services or access the Promega online catalog for the most up-to-date information on Promega products.

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